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Optimization of a whole-cell biocatalyst by employing genetically encoded product sensors inside nanolitre reactors

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Microcompartmentalization offers a high-throughput method for screening large numbers of biocatalysts generated from genetic libraries. Here we present a microcompartmentalization protocol for benchmarking the performance of whole-cell biocatalysts. Gel capsules served as nanolitre reactors (nLRs) for the cultivation and analysis of a library of *Bacillus subtilis* biocatalysts. The *B. subtilis* cells, which were co-confined with *E. coli* sensor cells inside the nLRs, converted the starting material cellobiose into the industrial product vitamin B2. Product formation triggered a sequence of reactions in the sensor cells: (1) conversion of B2 into flavin mononucleotide (FMN), (2) binding of FMN by a RNA riboswitch and (3) self-cleavage of RNA, which resulted in (4) the synthesis of a green fluorescent protein (GFP). The intensity of GFP fluorescence was then used to isolate *B. subtilis* variants that convert cellobiose into vitamin B2 with elevated efficiency. The underlying design principles of the assay are general and enable the development of similar protocols, which ultimately will speed up the optimization of whole-cell biocatalysts.

Secreted molecules represent by far the largest body of industrial biotechnology products and are therefore the focus of numerous molecule discovery and biocatalyst development efforts[1–3]. Metabolic engineering[4] studies focus on the redirecting of metabolic pathways towards the efficient formation of a number of key metabolites required for product synthesis by means of recombinant DNA technology. They often rely on (semi)rational or stochastic design approaches[5] that incrementally modify a production strain and thereby lead to large numbers of genetic variants. Most labs that work on metabolic engineering can easily generate libraries of up to 10^9 genetically different isolates when still operating at the millilitre scale. As any of these mutants may potentially feature the desired trait (for example, elevated productivity, yield or process robustness), powerful analysis methods to assess the catalytic properties of as many variants of the parent strain as possible are required. Assessment entails monoseptic growth of the respective variants in a single compartment, followed by some sort of quantification of the secreted product in the aqueous phase. Neither monoseptic reproduction of the strain nor product quantification is a trivial task and may require separation of the supernatant from the cells and slow chromatographic purification of the secreted product prior to spectrometric analysis. To this end, screening campaigns in industrial settings are frequently performed in microtitre plates, usually with the throughput capped at no more than 10^4 analysed variants per evolution round. To address this, microcompartmentalization approaches that feature a far higher throughput have been developed. Examples of this include qualitative assays that report on the presence of inhibitory antibodies secreted by single hybridoma cells[6] and lactic acid accumulated by cultivated *E. coli* strains[7]. Quantitative assays that report on the evolution of enzyme catalysts in *in vitro* systems[8,9] have also been designed. However, a method for the quantitative analysis of metabolites secreted by industrial whole-cell biocatalysts has not yet been reported. This is essential to deal with incremental improvements obtained in practical screening campaigns.

Biosensors[10,11] are well known for their high robustness, selectivity and sensitivity and have a long history as detectors in complex samples in the food, environmental and medicinal sectors. The design of new biosensors is now facilitated by the availability of increasingly sophisticated methods for the recruitment of aptamers that comprise RNA[12]. Similar to antibodies or enzymes, aptamers feature a high affinity for both small molecules and larger structures[13–17] such as proteins. Aptamers can, however, be converted into genetic actuators called riboswitches by combining them with another RNA sequence referred to as an expression platform[18,19]. These switches undergo conformational changes on ligand binding that result in a switch between states that are OFF (no translation of an adjacent open reading frame) and ON (translation occurs of an adjacent open reading frame encoding, for example, a green fluorescent protein (GFP)). The combination of highly specific riboswitches with the appropriate microbial sensor chassis thus allows the generation of highly specific microbial sensors[20–22]. Furthermore, if the nutrient requirements of producer and sensor strains are orthogonal—which can be achieved by either careful selection or simple gene knock-outs in the sensor strain—then the aptamer–riboswitch sensor is a robust indicator of product concentration directly within the same compartment, with no need to resort to laborious analytics. Instead, the level of secreted product is converted into an easily measurable signal that originates from the sensor strain.

Taking advantage of this sensing principle, here we identify *Bacillus subtilis* strains that secrete particularly high levels of vitamin B2 when using cellobiose as a carbon source (Fig. 1). Cellobiose is one of the main components of cellulosic biomass hydrolysates, which makes it a potential future feedstock for

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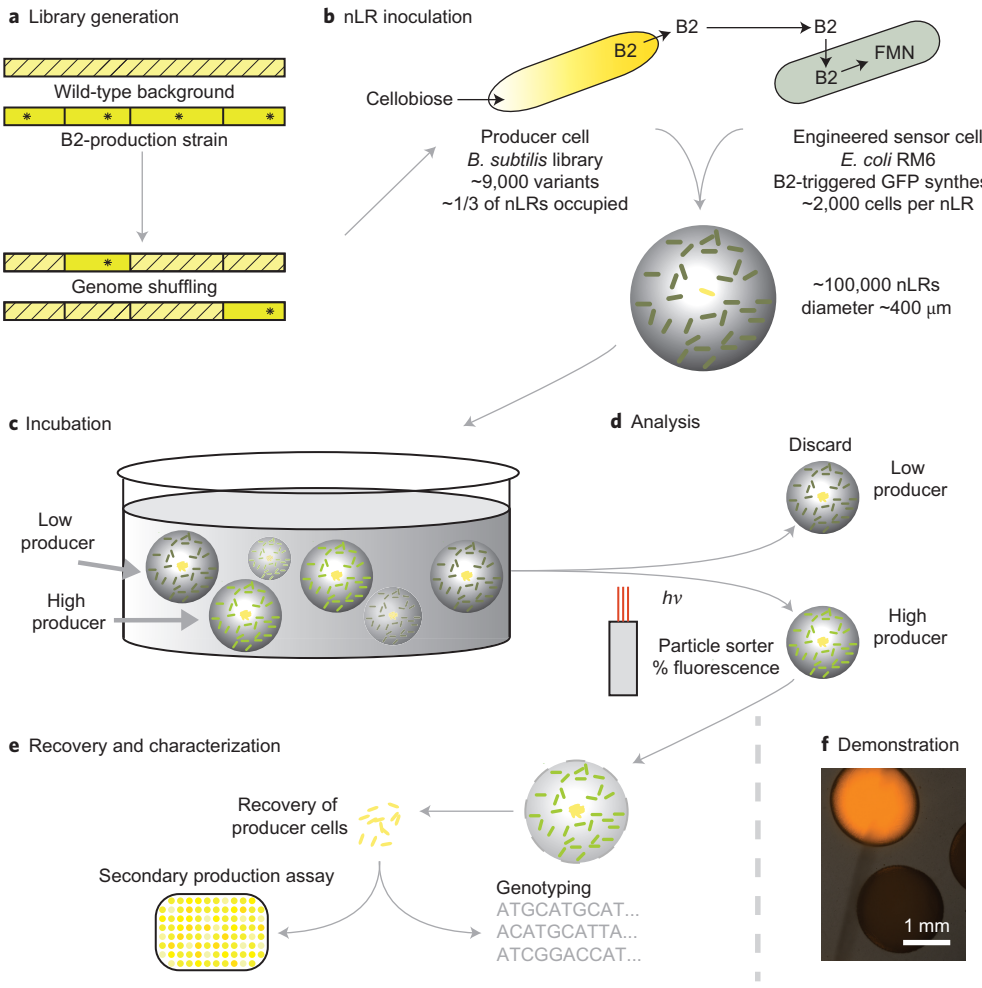

Figure 1 | Workflow for the optimization of a whole-cell biocatalyst performing the conversion of cellobiose into vitamin B2. a, A genome library was generated by cross-breeding of a *B. subtilis* wild type with an industrial isolate for the production of B2 from glucose. **b**, The resulting library of producer cells was inoculated in nLRs together with *E. coli* RM6 sensor cells by Poisson dilution adjusting for an average number of 0.3 for the library cells and 2,000 for the sensor cells. The sensor cells synthesize a GFP protein under the control of a riboswitch that bears the FMN-binding 21A aptamer and thus produce GFP in response to the FMN obtained from B2. **c**, The nLRs were soaked with a medium that contained cellobiose as the growth substrate for the library cells and galactose for the sensor cells. Incubation took place in a hydrophobic solvent to minimize B2 crosstalk. In the course of the incubation, the library cells grew into microcolonies that contained about 10,000 cells, whereas the sensor cells did not proliferate because of a thiamine auxotrophy. **d**, B2 secreted by a library colony is taken up by the co-contained sensors cells, which respond with the production of low (dark-green cells) or high (bright-green cells) levels of GFP. The nLRs were isolated by a fluorescence-assisted particle sorter and the most fluorescent fraction was isolated. **e**, The isolated producer cells were recovered from the nLRs and subjected to further characterization. **f**, Verification of the absence of the relevant vitamin B2 diffusion (crosstalk) under incubation conditions by using large-diameter nLRs. The nLRs were filled with culture medium with or without B2, suspended in the carrier phase and incubated. As demonstrated by the overlay of bright-field and fluorescence microscopy images (B2 in orange), B2 remains trapped, which thereby suggests minor crosstalk during the incubation. The particles had to be increased considerably over that of an nLR to allow imaging (see Methods for details).

industrial biotechnology products[23]. B2 secreted by a *B. subtilis* cell is taken up by metabolically active, but growth-arrested, *E. coli* sensor cells. The sensor cells then transform B2 into flavin mononucleotide (FMN) which binds with high affinity to a specifically developed hammerhead riboswitch, switching its conformation to ON and triggering GFP production in the sensor cells (Fig. 2) in a concentration-dependent manner. To perform the assay in a highly parallelized fashion, we developed a microcompartmentalization protocol[24,25] to confine the sensor cells together with a single *B. subtilis* cell inside nanolitre reactors (nLRs). The different *B. subtilis* strains were formed from a library of genome-shuffled producer cells. Crosstalk was circumvented by suspending the nLRs in a hydrophobic phase (Fig. 1) during growth and B2 production. This arrangement easily allows the primary analysis of 10^6 isolates per day.

Results and discussion

Isolation of an aptamer for the quantification of B2. We first focused on the identification of an aptamer[17] for the quantification of B2 (Fig. 2 and Supplementary Fig. 1). In prokaryotes, B2 is converted by housekeeping riboflavin kinases (RibF in the case of *E. coli*) to FMN, which then feedback-inhibits B2 synthesis via an FMN-sensitive riboswitch. Even though the system behaves with the characteristics of an OFF switch (which is undesirable for high-throughput experimentation, see below), we reasoned that it might be a good source of an aptamer. Once isolated, the switch characteristics of this aptamer could still be inverted (see below). We therefore investigated the 5′-untranslated region of the endogenous B2 synthesis operon[26] of *E. coli* (Supplementary Fig. 2) and, indeed, found a fragment that encoded for a putative FMN riboswitch[27]. We placed this fragment

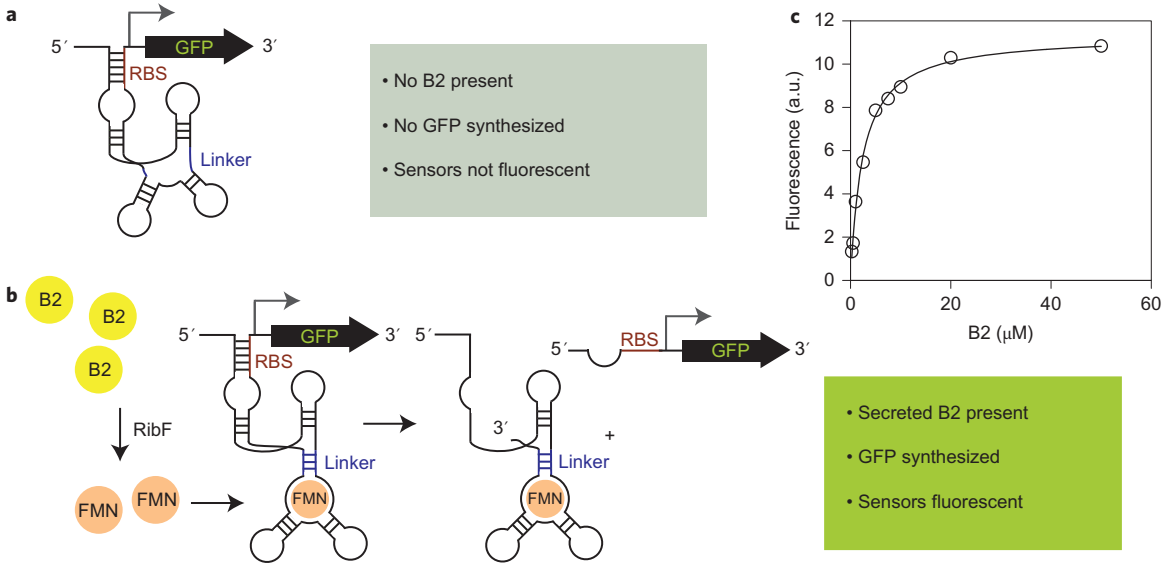

Figure 2 | B2 quantification in nLRs by genetically encoded sensors. a,**b**, *E. coli* RM6 sensor cells in their OFF (**a**) and ON (**b**) states. The cells translationally regulate GFP synthesis via a specifically tailored riboswitch that comprises the FMN aptamer 21A, an optimized connecting linker and a hammerhead ribozyme. In the OFF state (**a**), predominately formed in the absence of B2, the riboswitch is coiled in such a way that the ribosome binding site (RBS) remains buried and therefore inaccessible. The adjacent RNA sequence that encodes GFP is therefore not translated into protein. The transition to the ON state (**b**) is triggered by externally supplied B2. After its internalization, B2 is phosphorylated to FMN by the housekeeping enzyme RibF. FMN then binds to the aptamer and induces a conformational change of the entire riboswitch. As a consequence, self-cleavage is triggered, which culminates in the exposure of the previously buried RBS and, finally, in GFP synthesis. **c**, Dose–response curve obtained from the engineered riboswitch that bears the 21A aptamer expressed in *E. coli* RM6 sensor cells. The cells were grown in suspension and B2 was added to the indicated concentrations for the GFP induction. The experiment was performed in triplicate at a standard deviation of 1.3 to 3.3% for each data point (error bars are too small to be visible). a.u., arbitrary units.

together with the first 72 bases of the adjacent gene *ribB* in front of a *gfp* gene and transformed this construct into *E. coli* RM6 (Supplementary Fig. 2a,c). This cell line cannot produce B2 (because it has a deletion in the riboflavin synthase gene, *ΔribC*) and expresses the *ribM* gene encoding a B2-transport facilitator from *Corynebacterium glutamicum* (Supplementary Fig. 3)[28], such that externally provided B2 can be readily taken up and converted into FMN by the housekeeping riboflavin kinase. Indeed, the levels of GFP synthesized in *E. coli* RM6 containing the reporter construct were inversely correlated to the B2 concentration in the growth medium, which confirms that the selected sequence was functional as a riboswitch, even though one with an OFF characteristic (Supplementary Fig. 2b).

Inversion of switch behaviour. For high-throughput analyses, an ON switch would be much more desirable because it would then be possible to discriminate on the basis of an increasing signal (for example, fluorescence intensity) rather than a decreasing one. Based on sequence homology of the isolated ribozyme with the already identified FMN aptamers of other microbes[27], a putative 129 base pair (bp) aptamer region was fused to stems I and II of the fast-cleaving hammerhead ribozyme of *Schistosoma mansonii* via a 4 bp linker, thereby replacing stem III (Supplementary Fig. 1)[29]. In the presence of ligands, hammerhead ribozymes undergo autocatalytic cleavage that leads to the release and thus activation of an mRNA encoding, for example, for a marker gene and therefore, in principle, represent the desired ON switch behaviour. Critically, it was previously possible to obtain, by tailoring the linker, ribozyme variants with different response levels[18]. We therefore randomized the eight nucleotides of the linker region, placed the resulting population of hammerhead ribozyme variants in front of a *gfp* gene and introduced them into the strain RM6*ribM*. This library of putatively 65,536 (4^8) members was embedded in nLRs and the cells were expanded to microcolonies of approximately 10,000 cells at very low concentrations of externally supplied B2. Colonies that displayed fluorescence under these conditions (which suggests either a constitutively active or a hypersensitive riboswitch) were discarded after passage through a fluorescence-assisted particle sorter (Supplementary Fig. 4). The remaining fraction of nLRs contained all the non-fluorescent microcolonies and was then directly incubated in a rich medium supplemented with B2 concentrations close to the maximum B2 water solubility (250 μM). Strains that switch from non-fluorescent (under conditions of low B2) to highly fluorescent (under conditions of high B2) should bear, as an ON switch, a hammerhead ribozyme of the desired overall behaviour and, possibly, they should also bear suitable affinities (no signal at very low B2; high signal at B2 close to water saturation) and kinetics (activation in the time frame of the experiment).

In total, eight strains with such desirable characteristics were isolated and characterized in more detail in microtitre plate assays. The results indicated that the aptamer-bearing hammerhead ribozyme 2A1 performed best (Supplementary Fig. 5a,b) in terms of signal gain (a factor of ~15 between the OFF and ON states) and a half-maximal effective (external) B2 concentration of 3.6 ± 0.4 μM (Fig. 2). *In vitro* cleavage characterization experiments of the 2A1 riboswitch indicated that, similar to the riboswitch of *B. subtilis*[27], FMN indeed triggered self-cleavage, whereas B2 did not (Supplementary Fig. 6a,b). In addition, mutations known to disrupt the ligand binding of an FMN-specific switch isolated from *B. subtilis*[30] or to deactivate the self-cleavage mechanism of riboswitches[17] effectively prevented the generation of fluorescence in strain RM6 even at B2 concentrations close to water saturation (up to 100 μM (Supplementary Fig. 5c)), at which the parent 2A1 riboswitch displayed full activity. Consequently, *E. coli* RM6 synthesizing the 2A1 riboswitch effectively operates as an ON switch B2 sensor by importing B2 into the cytoplasm, converting it into

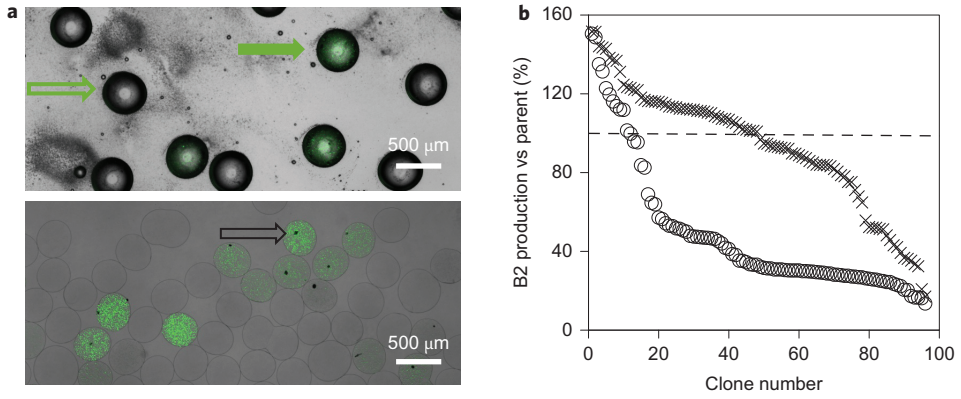

Figure 3 | Screening for high B2-producing *B. subtilis* strain variants. a, nLRs suspended in a hydrophobic solvent used as a mass-transfer barrier (upper panel) or in an aqueous buffer used during flow cytometric analyses (lower panel). All nLRs (including those that only displayed basal GFP fluorescence) contain ~2,000 *E. coli* RM6 cells each and statistically every third also contains a *B. subtilis* microcolony that comprises ~10,000 cells (the open black arrow indicates a representative colony). Consequently, two-thirds of all the nLRs contained either more than one or no *B. subtilis* colony. The nLRs display different degrees of fluorescence that range from non-fluorescent (open green arrow) to highly fluorescent (filled green arrow), indicating the different B2 amounts secreted by the encased *B. subtilis* cells. **b**, Final B2 concentrations of *B. subtilis* clones isolated in the course of the nLR screening versus a randomly selected control measured in 24-well plate assays on cellobiose. The data are normalized to the parent strain (itself already an industrial B2-production strain) used for the construction of the *B. subtilis* library (the dashed line indicates 100%). B2 titres of 96 *B. subtilis* variants selected after incubation in the nLRs (X) were compared with randomly selected *B. subtilis* B2 variants (O).

FMN and translationally regulating GFP synthesis via the optimized FMN-specific hammerhead ribozyme 2A1. This design strategy is modular and should be generally amenable to other ligands for which aptamers are available.

Generation of a library from a *B. subtilis* industrial B2 producer. Next, we generated a library based on *B. subtilis* BS4511, a strain previously used by industry to manufacture B2 from glucose. In this strain, the flux of B2 to FMN is lowered because of a down-regulation of the riboflavin kinase (*ribC* in the *B. subtilis* nomenclature) that results from the *ribC*820 mutation and, as a consequence, FMN does not accumulate to sufficiently high amounts to down regulate the B2 operon and therefore B2 synthesis[31]. However, the strain also possesses more than 100 additional poorly characterized mutations at different loci, which accumulated in the course of repetitive cycles of mutation, screening and selection performed over a decades-long history of strain development. Together, these mutations led to an additional increase in the B2 yield, but the contribution of the single mutations remains unclear. We reasoned that only a subset of them is responsible for the observed high B2 levels, whereas others are at best superfluous or, more probably, counterproductive.

Consequently, we aimed at the elimination of such counterproductive mutations by replacing them by their wild-type counterparts. To do so, we further developed and used a genome-shuffling method designated as a lysed protoplast transformation[32]. By 'crossing' the riboflavin production strain with wild-type *B. subtilis* we obtained a library of approximately 9,000 members. The sequence of the genomic DNA was determined for eight randomly selected clones by using the Illumina (Solexa) Genome Analyzer II technology (GATC Biotech AG). Based on these results, each transformant bore approximately ten visible crossovers during which about one-third of the non-mutated loci were transferred from the wild type to the high-producing receiving strain. Consequently, we obtained a highly diverse library of strains, in each of which a distinct subset of the large number of mutations of unknown effect in the parent strain was replaced. This library was next evaluated in view of the B2-production performance of its members on cellobiose, which could be a cheap next-generation carbon source for B2 production.

Screening of B2-producing *B. subtilis* libraries. To ensure library coverage of more than 95% in the subsequent analyses[33], 30,000 single library cells ('producers') and approximately 60,000,000 sensor cells ('sensors') were encapsulated within 100,000 nLRs, so that each nLR could be expected to contain 2,000 sensors and 0.3 producers. These are, of course, average numbers because of the random distribution of cells into the nLRs according to Poisson. An average occupation of 0.3 ensures a high frequency of nLRs that contain exactly one library cell, and a high degree of occupation such as 2,000 ensures that there is little variance in the number of sensor cells per nLR.

The competition between producers and sensors for evaluation-critical nutrients was prevented by ensuring orthogonal carbon sources: whereas the *B. subtilis* producers were grown on cellobiose (but were unable to grow with galactose) for B2 production, *E. coli* was provided with galactose (but was unable to grow with cellobiose) to enable GFP synthesis and intracellular conversion of B2 into FMN. The growth of the sensors after accumulation of B2 remained impossible as RM6*ribM* contains a *thiH* mutation and thus requires thiamine for growth[34], which was not provided.

The inoculated nLRs with cellobiose- and galactose-containing media were incubated for 40 hours in a mixture of heavy mineral oil and surfactants required to prevent B2 exchange between nLRs (Fig. 3). During this time, the producer cells expanded to microcolonies of about 10,000 cells and concomitantly secreted B2, which in turn triggered GFP synthesis in the co-localized sensors (Figs 1 and 2). Once the sensors had recorded the level of produced B2 to which they had been exposed, the two-phase suspension could be safely broken and oil and surfactants removed. The nLRs displayed different degrees of fluorescence and were resuspended in an aqueous buffer used as the sample fluid during fluorescence-assisted particle analysis and sorting into individual microtitre plate wells. We retained the 96 most-fluorescent nLRs (Supplementary Fig. 7), dissolved the hydrogel, recovered the producers and proliferated and genotyped them.

Characterization of the isolated variants. Afterwards, the isolated producer strains were subjected to a secondary production assay routinely used by DSM for the characterization of B2-secreting *B. subtilis* isolates. The assays were performed in 24-well microtitre plates and the performance of the isolated producers

was compared to that of a control population of 96 randomly picked clones isolated from agar plates immediately after plating of the genome-shuffled library. Genotyping indicated a ratio of the attenuating *ribC820* mutation and the *ribC* wild-type allele of 19 to 74 in the randomly selected and of 89 to four in the nLR-selected fraction (in both populations one clone could not be genotyped). This suggests a similar importance of the *ribC* attenuation for B2 overproduction on glucose and on cellobiose. The results obtained from the characterization of the isolated clones in an orthogonal microtitre plate assay indicated that 56 of the nLR-selected clones did not exceed the performance of the parent production strain used as a starting point for the generation of the library. As we very rarely, but consistently, observed agglomeration of smaller fractions of the nLRs during incubation, these strains may have been isolated from nLRs that obtained additional substrates via cross-feeding from an adjacent nLR with no producer. Still, nLR-selected strains performed considerably better than randomly selected strains, as indicated by the fact that 40 out of the 96 nLR-selected producers excelled over the parental producer strain but only ten did so in the control. Clearly, a high-throughput technology for the quantitative analysis at the nanolitre scale in combination with a modular sensor-design concept is an efficient way to identify strains for high B2 production.

Conclusions

Here we present a high-throughput method to discriminate between efficient production strains with modest changes in their genotype. Secreted products can be evaluated quantitatively based on a modular engineered ribozyme, which suggests that the method could, in principle, be of broad applicability by following a workflow of: (1) aptamer engineering (for example, via SELEX) or isolation via *in silico* data mining, (2) construction of ribozymes for the regulation of one or more marker genes, (3) optimization of the ribozyme response levels by means of linker-region optimization in an appropriate host, (4) orthogonalization of the nutrient requirements of the producer and the sensor strains, (5) co-incubation of producer and sensor in nLRs and (6) determination of the synthetic power of the whole-cell biocatalyst variants on the basis of the signals generated by the sensors co-localized in the nLR. All the required components are generic: appropriate transport proteins allow passage of the product of interest over the membrane of the sensors[35], and many currently relevant large-scale biotechnology products (see Supplementary Table 1) can cross the membrane even without a facilitator. Similarly, translational or transcriptional regulators based on the RNA encoding product-sensing protein are likely to be either already available or will be obtained rapidly by engineering. Thus, the described method will be broadly applicable for strain-development campaigns and may possibly also find application in the pharmaceutical product-manufacturing segment.

Methods

Production of nLRs. Encapsulation of *B. subtilis* and *E. coli* cells into the nLRs was performed with a laminar jet break-up encapsulator (Nisco Engineering AG). An aliquot of 15 g of sterile filtered 2.5% (w/v) aqueous sodium alginate (Sigma-Aldrich) was mixed with 3 ml 0.9% (w/v) sodium chloride that contained the cells for encapsulation. Droplet formation was done with a 150 µm nozzle at a flow rate of 3.7 ml min^{-1} and a production frequency of 1,050 Hz. The droplets were collected in a continuously stirred beaker filled with 100 mM calcium chloride and allowed to mature for 30 minutes. The resulting nLRs had a diameter of approximately 500 µm, which corresponded to a volume of about 65 nl.

Construction of the *B. subtilis* library. The industry strain *B. subtilis* 4511 efficiently converts glucose into B2 because of a number of mutations on the chromosome. *B. subtilis* BS4842 is a B2 auxotrophic mutant of the reference strain *B. subtilis* 168, which has been deleted for all the genes that encode for enzymes of the final B2 pathway (*ribDEAHT* in the notation of *B. subtilis*) and harbours a spectinomycin-resistance gene (at the *amyE* chromosomal locus) to allow counterselection against regenerated protoplasts of BS4511. To create a mutant library of *B. subtilis* strains in which each genome is a unique combination of the initial pool of mutations, we applied the transformation of competent *B. subtilis* by DNA in protoplast lysates as described previously[32]. Briefly, competent cells of BS4842 were prepared and transformed with protoplasted DNA of strain BS4511. The mutants that recovered the ability to grow without B2 supplementation because of the recombination of the engineered *ribDEAHT* operon from BS4511 DNA were selected on Spizizen's minimal medium[36] supplemented with roseoflavin (a B2 antimetabolite that efficiently suppresses the growth of B2 auxo- and bradytroph isolates) at 100 µg ml^{-1}.

Screening of *B. subtilis* library. The *B. subtilis* library cells and riboswitch-containing *E. coli* sensors were co-encapsulated in nLRs at a frequency of occupation of 0.3 and 2,000, respectively. After production, the compartments were soaked with bead minimal medium (per litre: 0.2 g $(NH_4)_2SO_4$, 1.4 g K_2HPO_4, 0.6 g KH_2PO_4, 20 mg $MgSO_4$, 12.5 mg $MgCl_2$, 0.5 mg $CaCl_2$, 50 mg yeast extract, 1 × trace element solution[37], 50 mg l^{-1} D-cellobiose and 5 g l^{-1} D -galactose for ten minutes to fill the nLRs with the medium. The nLRs were isolated from the medium by sieving (mesh size 100 µm) and placed into heavy mineral oil that contained 2% ABIL 90 and 0.05% Tween 20. After thorough mixing, the nLRs were incubated in two 100 mL plastic containers with screw caps (TP51-011 from Gosselin for 40 hours at 37 °C on a shaker (Infors HT, 200 revolutions per minute). At the end of the cultivation, the nLRs were separated from the hydrophobic phase by centrifugation (ten minutes, 72*g*) and washed with 10 mM $CaCl_2$ containing 0.1% Tween 20. The most fluorescent nLRs were isolated by particle sorting with a COPAS Plus (Union Biometrica) and dispensed into individual wells of a 96-well plate. The addition of 200 µl tenfold concentrated bead minimal medium that contained, in addition, 1 g l^{-1} D-cellobiose and 100 mg l^{-1} spectinomycin dissolved the nLRs and allowed the *B. subtilis* strains, but not the sensor cells, to grow. After incubation overnight at 37 °C the individual strains were reisolated on tryptose blood agar and single colonies were taken for further characterization.

B2 assay in a 24-well format. Precultures were run overnight in 96 deep well plates at 37 °C in VY broth (veal infusion 25 g l^{-1}, yeast extract 5 g l^{-1}) supplemented with spectinomycin (100 µg ml^{-1}). The next day, 50 µl of the preculture were used to inoculate 3 ml of screening medium (K_2HPO_4 14 g l^{-1}, KH_2PO_4 6 g l^{-1}, $(NH_4)_2SO_4$ 2 g l^{-1}, $MgSO_4{\cdot}7H_2O$ 0.2 g l^{-1}, $MgCl_2{\cdot}6H_2O$ 0.125 g l^{-1}, $CaCl_2$ 5.5 mg l^{-1}, $MnCl_2{\cdot}4H_2O$ 1 mg l^{-1}, $ZnCl_2$ 1.7 mg l^{-1}, $CuCl_2{\cdot}2H_2O$ 4.3 mg l^{-1}, $CoCl_2{\cdot}6H_2O$ 0.6 mg l^{-1}, $Na_2MoO_4{\cdot}2H_2O$ 0.6 mg l^{-1}, $Fe(III)Cl_3{\cdot}6H_2O$ 1.35 mg l^{-1}, yeast extract 50 mg l^{-1}, pH 6.8) that contained glucose 1 g l^{-1} and cellobiose 9 g l^{-1}. Cultures were run during 48 hours in 24 deep well plates (39 °C) with an assay used routinely by DSM to benchmark the performance of B2-producing *B. subtilis* clones and generally characterized by a coefficient of variance (CV) <10%. Interday performance was monitored by dedicating one well of each of the four plates to the B2 reference strain *B. subtilis* BS4511, also used for the construction of the library. The results indicated a CV of >5% (105, 104, 97 and 94% of the parent). An aliquot of 500 µl of the 48 hour culture was mixed with 40 µl of a 4 M NaOH solution to solubilize B2 crystals and neutralized with 460 µl of a 1 M potassium phosphate buffer (pH 6.8). The concentration of B2 was assayed by measuring the absorbance at 444 nm at an appropriate dilution. A $D_{444\,nm}$ of one unit corresponds to 33.05 mg l^{-1} of B2. The B2 yield is given in percent of the yield of the BS4511 reference strain incubated under the same assay conditions.

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Author contributions

A.M. developed the sensor strains and the aptamers. R.P. developed the incubation and COPAS protocols. K.B., A.M. and R.P. performed the screening. S.Po. generated the *B. subtilis* library and characterized the isolated strains. All the authors discussed the results and commented on the manuscript. All the authors assisted in co-writing the paper.

Additional information

Supplementary information is available in the online version of the paper. Reprints and permissions information is available online at www.nature.com/reprints. Correspondence and requests for materials should be addressed to M.H.

Competing financial interests

H.P.H. and S.Po. are with DSM NP, which co-sponsored the development. A.M., R.P., S.Pa. and M.H. are affilliates of FGen GmbH, which develops similar protocols.